

Land-use change to bioenergy: grassland to short rotation coppice willow has an improved carbon balance

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Abstract

The effect of a transition from grassland to second-generation (2G) bioenergy on soil carbon and greenhouse gas (GHG) balance is uncertain, with limited empirical data on which to validate landscape-scale models, sustainability criteria and energy policies. Here, we quantified soil carbon, soil GHG emissions and whole ecosystem carbon balance for short rotation coppice (SRC) bioenergy willow and a paired grassland site, both planted at commercial scale. We quantified the carbon balance for a 2-year period and captured the effects of a commercial harvest in the SRC willow at the end of the first cycle. Soil fluxes of nitrous oxide (N_2O) and methane (CH_4) did not contribute significantly to the GHG balance of these land uses. Soil respiration was lower in SRC willow ($912 \pm 42 \text{ g C m}^{-2} \text{ yr}^{-1}$) than in grassland ($1522 \pm 39 \text{ g C m}^{-2} \text{ yr}^{-1}$). Net ecosystem exchange (NEE) reflected this with the grassland a net source of carbon with mean NEE of $119 \pm 10 \text{ g C m}^{-2} \text{ yr}^{-1}$ and SRC willow a net sink, $-620 \pm 18 \text{ g C m}^{-2} \text{ yr}^{-1}$. When carbon removed from the ecosystem in harvested products was considered (Net Biome Productivity), SRC willow remained a net sink ($221 \pm 66 \text{ g C m}^{-2} \text{ yr}^{-1}$). Despite the SRC willow site being a net sink for carbon, soil carbon stocks (0–30 cm) were higher under the grassland. There was a larger NEE and increase in ecosystem respiration in the SRC willow after harvest; however, the site still remained a carbon sink. Our results indicate that once established, significant carbon savings are likely in SRC willow compared with the minimally managed grassland at this site. Although these observed impacts may be site and management dependent, they provide evidence that land-use transition to 2G bioenergy has potential to provide a significant improvement on the ecosystem service of climate regulation relative to grassland systems.

Keywords: carbon balance, bioenergy, climate regulation, ecosystem services, grassland, greenhouse gas, soil carbon

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Introduction

Dedicated second-generation (2G) nonfood feedstocks offer an opportunity to provide biomass for bioenergy-derived heat, electricity and biofuels without competing with land for food (Dornburg *et al.*, 2010; Stoof *et al.*, 2015). However, evidence is still required to support this assertion, particularly with respect to soil properties (Kort *et al.*, 1998), greenhouse gas (GHG) emissions (see refs within Rowe *et al.*, 2009) and a whole basket of associated ecosystem services (Holland *et al.*, 2015). Although recent reports suggest that energy and food may be produced in a multifunctional landscape in a sustainable way (Manning *et al.*, 2015; Souza *et al.*, 2015), many of these positive effects are dependent on land management, vegetation type, and in particular, the land-use change (LUC) implemented when the

bioenergy crop is planted (Milner *et al.*, 2015). It is therefore important to consider how these crops will be placed within the landscape (Dauber *et al.*, 2010) and the impacts of particular land-use transitions on ecosystem services, of which climate regulation is of outstanding importance (Anderson-Teixeira *et al.*, 2012). In 2013, $51 \times 10^3 \text{ ha}$ (0.8% total arable land) were used to grow bioenergy in the UK (DEFRA, 2014) and, at the same time, it is estimated that there are still $3.5 \times 10^6 \text{ ha}$ of land currently available to grow bioenergy crops without impacting food production (Lovett *et al.*, 2014), with estimated yields ranging from 6 to $12 \text{ t ha}^{-1} \text{ yr}^{-1}$ for SRC willow (Hastings *et al.*, 2014). Adoption of bioenergy will inevitably result in large scale LUC; therefore, it is important to consider which land classes are most suited to the conversion to minimize environmental damage and competition with food crops.

Land-use change, irrespective of crop type, may have many direct consequences on climate regulation, such as altered GHG emissions (IPCC, 2007a), changes in soil

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carbon (Guo & Gifford, 2002) as well as impacts on other ecosystem services and biodiversity (Sala *et al.*, 2000). Additionally for bioenergy crops, the impacts of indirect land-use change (iLUC; Searchinger *et al.*, 2008; Melillo *et al.*, 2009; Finkbeiner, 2014) and those of quantifying the counterfactual land use (DECC, 2014; Mathews *et al.*, 2014) are increasingly recognized and considered in land-use conversions. St. Clair *et al.* (2008) found that former land use is the most important consideration determining whether a transition to 2G bioenergy will result in a net source or net sink of carbon. A number of studies and meta-analyses have suggested that, although dependant on site, LUC from arable cropping to 2G bioenergy is most likely to result in neutral or net increases in soil carbon (Dimitriou *et al.*, 2012; Don *et al.*, 2012; Harris *et al.*, 2015; Qin *et al.*, 2015). Similarly, reductions in other GHG emissions have also been reported for LUC from arable to 2G bioenergy (Drewer *et al.*, 2012; Gauder *et al.*, 2012; Zona *et al.*, 2013a; Palmer *et al.*, 2014), a proportion of which is attributable to change in management and land-use intensity. However, there is much more uncertainty surrounding the effects of LUC from grassland to 2G bioenergy crops (Harris *et al.*, 2015; Qin *et al.*, 2015), partly reflecting the considerable variability that is found amongst grassland types with significant differences in management which can dictate GHG balance (Soussana *et al.*, 2010). Although grasslands may be managed to encourage a carbon sink (defined here as an ecosystem in which the net gain of carbon is greater than the net loss; Smith, 2014), other management practices such as fertilizer addition and grazing may lead to large emissions of nitrous oxide (N_2O) and methane (CH_4). Ciais *et al.* (2010) suggested that emissions of N_2O and CH_4 following management practices may offset approximately 70–80% of the net carbon sink in European grasslands. This indicates that conversion to 2G bioenergy cropping may result in additional GHG savings. Moreover, Styles & Jones (2007) demonstrated that initial cultivation emissions associated with LUC from grassland to SRC willow could be offset by GHG emissions savings from replacing fossil fuel usage. The timescale for this ‘payback’, as calculated from current research is uncertain, varying between 0 and 423 years depending on former land use, management and bioenergy crop cultivated (Fargione *et al.*, 2008; Don *et al.*, 2012; Ter-Mikaelian *et al.*, 2015).

Two limitations are apparent when considering much of the literature in current LUC and bioenergy research. The first is that many studies rely entirely on modelled data with extremely limited or no validation (Cherubini *et al.*, 2009) and this is worrying, given that outputs from such models, often parameterized for non-bioenergy ‘exemplar’ arable, grass and tree ideotypes,

may be used to develop sustainability criteria and policy instruments (Creutzig *et al.*, 2012; Buchholz *et al.*, 2014). Secondly, when empirical data have been captured for model validation, they have often been small research-scale plots of limited commercial relevance (e.g. Nikiema *et al.*, 2012; Zatta *et al.*, 2014). Additionally, there are methodological considerations which may affect the conclusions drawn about LUC, such as soil sampling depth (Dolan *et al.*, 2006; Blanco-Canqui & Lal, 2008) and calculation of soil carbon stocks using a fixed depth method (Walter *et al.*, 2015).

Given the need for empirical data, which is critical for LUC evaluation and model validation, here we present the results from a paired-site evaluation of LUC to bioenergy. The aim of this study was to quantify the impacts of a LUC at commercial scale from a grassland with limited management intervention, to that of SRC willow and to quantify the ecosystem GHG balance of this change 7 years after conversion. During 3 years of measurement, the SRC willow was harvested at commercial scale, and the impact of this activity on GHG balance and whole ecosystem carbon balance was also quantified. These findings will add to our understanding of the effects of LUC to bioenergy in temperate climates and contribute to the parameterization and testing of models to predict effect out to future climates.

Materials and methods

The aim of this side-by-side comparison was to develop an intensive data set for all components of the ecosystem GHG balance from a commercial plantation over a period of 3 years, including bioenergy SRC harvest. Figure 1 outlines the different components which were measured to assess the ecosystem GHG balance. The experimental set-up was established in November 2011 and measurements continued through until December 2014 (see Fig. S1 for experimental timeline).

Site description and management

This study was conducted in the south of England (50°58'N, 0°27'W) in an established SRC willow plantation (8.1 ha) and permanent grassland with low inputs (7.4 ha).

Mixed commercial genotypes of SRC willow were planted in June 2008 on a grassland field, previously defined as set-aside (2000–2007) at a density of 15 000 stems ha^{-1} in double rows with distances of 0.75 m in the row and 1.4 m between the rows (Forestry Commission, 2002). Prior to planting, the site was ploughed to 0.25 m in September 2007 and treated with herbicide (1.6 kg ha^{-1} glyphosate) and insecticide (0.75 kg ha^{-1} chlorpyrifos). In April 2008, the site was power harrowed to 0.10 m depth and there was a further application of herbicide (1.6 kg ha^{-1} glyphosate in June 2008). At pre-emergence the site was treated with herbicides (0.25 kg ha^{-1} isoxaben, 1.5 kg ha^{-1} pendimethalin) and insecticide (0.75 kg ha^{-1} chlorpyrifos). The

Net Biome Production (NBP):

The difference between Net Ecosystem Productivity (NEP, negative sign of NEE¹) and the carbon removed through harvest. A negative NBP indicates a release to the atmosphere and a positive NBP indicates an uptake by the ecosystem.

**Harvested Biomass:**

Carbon contained within biomass which is harvested. SRC harvest takes place every 4th year through a cut and chip mechanism. Grass is mowed annually and left in situ.

Litterfall

Leaf litter which falls from the trees to the ground. Estimated with litter traps.

**NBP****Gross Productivity(GPP):**

The fixation of carbon by autotrophic organisms i.e. plant material. Partitioned from NEE.

GPP**R_{eco}****NEE****Ecosystem Respiration(R_{eco}):**

The net respiration by both autotrophic and heterotrophic organisms in the ecosystem. Partitioned from NEE.

**Net Ecosystem Exchange (NEE):**

The CO₂ exchange between the ecosystem and the atmosphere, measured using the eddy covariance technique.

Primary Production:

The fixation of carbon by autotrophic organisms i.e. plant material. Partitioned from NEE.

Litterfall**Soil respiration:**

Sum of CO₂ respired by roots (autotrophic respiration) and by microbes (heterotrophic respiration). Measured by static and dynamic soil chambers

**Soil N₂O and CH₄:**

Fluxes of nitrous oxide (N₂O) and methane (CH₄) from soils. Measured using static soil chambers.

**Soil carbon:**

Carbon contained within the soil. Estimated by dry combustion.

Belowground biomass:

Carbon contained within the roots. Measured in grassland and estimated in SRC willow



Fig. 1 Measurements taken to establish whole ecosystem greenhouse gas balance showing the main flows of carbon through the ecosystem. Crop shown represents short rotation coppice willow but cycle is applicable to any vegetation type.¹Reichstein *et al.* (2005).

SRC willow was cut back in March 2009, further treated with herbicide (2.3 kg ha⁻¹ aminotriazole) and then underwent a rotation of 5 years prior to harvest in April 2014.

The grassland site was enlisted in the set-aside scheme until 2004 and was maintained as low input grassland thereafter. The site was a mixed grassland including *Lolium* spp., *Schedonorus* spp., *Dactylis* spp. and other cultivated species. There were no inputs to the site other than an addition of a total of 10 t of manganese lime across the site in April 2011. Management was variable year to year, with grazing by sheep once per year (2–4 weeks), or if this did not occur, the grass was mown to control grass height. During the experiment, the site was grazed for 2 weeks in 2012 and the grass was mown in August/September in 2013 and 2014. Mowed grass was left on the site.

Mean annual rainfall at the sites is 794 mm, and mean annual temperature is 11.0 °C (1960–2010; Met Office, 2015). The soil is the same at both sites, silt loam (Table 1) with a pH of 5.5. Root exploration depth was 0.30 m in grassland, with the majority of root biomass found in the top 0.15 m and SRC willow roots were found to 1 m, with the majority of biomass in the 0.50–1.00 m horizon (Table S1). The dominant wind direction is from the southwest; therefore, eddy covariance towers were established in the north-easterly corner of the

Table 1 Soil texture for grassland, short rotation coppice willow and initial grassland site

Site	Depth (cm)	Clay	Silt	Sand	Soil type
		(%)	(%)	(%)	
SRC willow	0–15	7.38	59.44	33.19	Silt loam
	15–30	6.93	60.06	33.02	Silt loam
Grass	0–15	5.54	65.27	29.19	Silt loam
	15–30	14.06	62.79	23.15	Silt loam
Initial grassland	0–15	6.43	69.62	23.94	Silt loam
	15–30	15.26	66.69	18.04	Silt loam

grassland and SRC willow in order to ensure enough fetch (Fig. 2).

Micrometeorological measurements

A meteorological station was installed in SRC willow in August 2011 and in grassland in November 2011 (Fig. S1). Each station measured soil temperature and heat flux at three depths (5, 10 and 15 cm; TCAV; Campbell Scientific, Logan, UT, USA; HFP01SC heat flux plates; Campbell Scientific), soil water

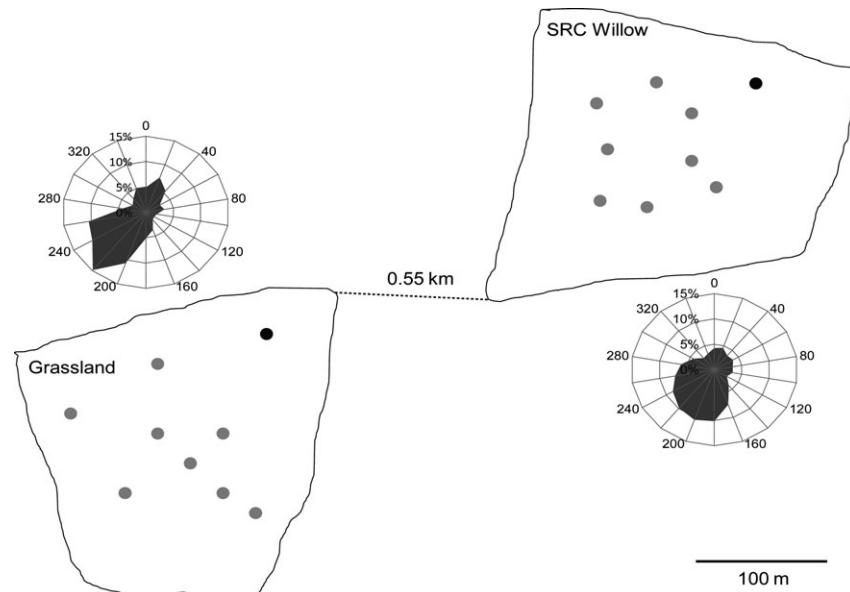


Fig. 2 Site maps of grassland and short rotation coppice willow, including wind rose for each site showing a predominant south-westerly wind. Black circle indicates location of eddy covariance tower and meteorological station. Grey circles indicate experimental plots where soil greenhouse gas, litter fall, litter decomposition measurement were taken. 100 m rule indicated for scale.

content using time-domain reflectometers (CS616; Campbell Scientific), incoming photo flux density (SKP215 quantum sensor; Skye Instruments, Powys, UK), net radiation (NR-LITE; Kipp and Zonen, Delft, the Netherlands), air temperature and humidity (HMP155A; Vaisala, Vantaa, Finland). Additionally, precipitation (52203; Young, Traverse City, MI, USA) and wind speed and direction (05103-5; Young) were measured at the SRC willow site only. At both stations, variables were measured at 0.1 Hz and then collected and averaged half-hourly using a CR1000 datalogger (Campbell Scientific). The 50-year (1960–2010) average monthly temperature and rainfall for the region were obtained from the UK Met Office (Met Office, 2015).

Soil GHGs fluxes

Eight plots were established in random locations in the SRC willow and grassland in November 2011 to measure soil GHGs, soil chemistry, aboveground and belowground biomass, litter fall and litter decomposition (Fig. 2). Randomization took place within a few metres of field edges to avoid any edge effects. Within these plots, soil CO₂ efflux was measured at monthly intervals using a portable chamber (SRC-1; PP Systems, Amesbury, MA, USA) coupled with an IRGA (EMG-4; PP Systems). Every effort was made at each sampling date to avoid the inclusion of significant amounts of vegetation in the sampling chamber, since this would reflect both plant shoot as well as soil and root efflux. However, small amounts of shoot vegetation remained inside the chamber, and therefore, soil CO₂ flux may be overestimated. Air temperature, soil temperature (stab probe; Testo, Alton, Hampshire, UK; 0–10 cm) and soil moisture (Theta probe; Delta-T, Burwell, Cambridge, UK; 0–6 cm) were also measured around the chamber at the time of sam-

pling. As soil temperature is generally a good predictor of soil respiration, annual soil respiration was computed using an exponential function between monthly soil respiration data (SR) and continuous soil temperature data (T_{soil}) measured at each weather station (Raich & Schlesinger, 1992; Raich *et al.*, 2002):

$$\text{SR} = a \times \exp^{b \times T_{\text{soil}}}.$$

At each of the eight sampling locations, N₂O and CH₄ soil fluxes were measured using closed vented static chambers (Smith & Mullins, 2000) made of PVC base rings (8 cm high with a diameter of 40 cm), inserted in the soil to 5 cm depth, and chamber lids (20 cm high with a diameter of 40 cm). To determine GHG fluxes, headspace gas (10 ml) was sampled from a self-sealing septa in the chamber lid using gas-tight syringes, at 0, 15, 30 and 50 min after closure; it was immediately stored in pre-evacuated gas-tight vials (3 ml; Labco Ltd, Lampeter, Ceredigion, UK). Gas samples were analysed on a PerkinElmer Autosystem XL Gas Chromatograph (GC) fitted with a flame ionization detector for CH₄ and an electron capture detector for N₂O. All results were calibrated against certified gas standards (BOC, Guildford, UK; Case *et al.*, 2014). N₂O and CH₄ flux rates were determined by linear regression of the four sampling time points for each chamber and by applying a temperature and pressure correction (Holland *et al.*, 1999). The analytical precision of the GC for standards at ambient concentration was approximately 2%, using two standard deviations as a measure of mean error. Sampling for soil GHG fluxes took place every month, from November 2011 until December 2014 (Fig. S1). Sampling of the grassland initially took place in a smaller grassland site from November 2011 until August 2012, when sampling was moved to another larger site (to accommodate eddy covariance equipment). Grassland sites were both

sampled for GHG fluxes for the next 3 months to compare fluxes and there was no significant difference between the sites ($t_{(4)} = -0.06$, $P = 0.95$). Non-CO₂ GHG fluxes were first converted into CO₂ equivalents using the global warming potentials over a 100-year horizon of 298 for N₂O and 25 for CH₄ and then to carbon equivalents using a conversion factors of 0.2727 (IPCC, 2007b). Linear interpolation between measurements dates (i.e. trapezoidal integration) was used to compute annual cumulative GHG fluxes.

Six (two per plot: one root excluded, one total respiration) automated soil chambers were also established in the SRC willow in February 2012 (Ventura *et al.*, 2015). These chambers measured soil CO₂ flux every 4 h, and three of the chambers were placed in root exclusion chambers to allow the partitioning of autotrophic and heterotrophic respiration. Data from automated chambers were used to validate periodic measurements.

Soil analysis

Soil carbon was measured at 0–30 cm (15 cores) and to 1 m depth (three cores) in both grassland and SRC willow (and initial grassland). Samples were only taken once during the experiment in October 2012. Five plots were randomly selected in each field; from each of these plots, three within-plot soil cores were taken using a split-tube soil sampler (Eijkkelkamp Agri-search Equipment BV, Giesbeek, the Netherlands) with an inner diameter of 4.8 cm to a depth of 30 cm. This gave a total of 15 spatially nested samples per field, accounting for both field-scale (between sampling plots) and plot-scale (cores within plots) variability. One of the five sampling plots was randomly selected and three additional 1 m cores were taken. In the case of both the 1 m and 0–30 cm core, one core was taken from the centre of the plot, with two further cores taken at distances of 1 and 1.5 m in random compass directions from the centre. The 1 m cores were taken using a window sampler system with a 4.4 cm cutting diameter (Eijkkelkamp Agri-search Equipment BV), allowing a full 1 m core to be extracted and subsequently transported in one section. If coring to the full depth was not possible, for example when large stones or bedrock were encountered, the precise depth of the cored hole was recorded (see Rowe *et al.*, 2016, for full methods). Fresh soil was sieved to 2 mm before being frozen at -80 °C and subsequently freeze-dried for minimum of 24 h. A subsample of the freeze-dried soil (20–30 ml) was milled to a fine powder in a ball mill (Planetary Mill; FRITSCH, Idar-Oberstein, Germany). A 200 mg subsample of the milled soil was used for the assessment of carbon concentration using an elemental analyser (Leco Truspec CN, Milan, Italy). Total soil carbon stock for the 0–30 and 0–100 cm fractions was calculated on an equivalent soil mass basis (Keith *et al.*, 2015).

Aboveground and belowground biomass and net primary production (NPP)

Aboveground biomass. In SRC willow, aboveground biomass was estimated from the stem : volume index (Pontallier *et al.*, 1997) which was calculated for all shoots of 160 stumps distributed in eight plots using stem diameter (22 cm from

ground height; Rae *et al.*, 2004) and dominant stem height. Nondestructive sampling took place every year in winter during the experiment (Fig. S1). Destructive sampling of SRC willow was also conducted prior to commercial harvest in November 2013, to allow an estimation of actual biomass from stem : volume index values. A linear regression of stem:volume index against fresh weight allowed estimation of total dry weight (kg tree⁻¹) from trees which were nondestructively sampled. Total carbon contained in aboveground biomass was calculated by assuming that the amount of carbon contained in woody biomass was approximately 49.3 ± 1.2% (mean ± SD), calculated from an assessment of measured values in the literature for SRC (Fahmi *et al.*, 2007; Bridgeman *et al.*, 2008; Sannigrahi *et al.*, 2010; Gudka, 2012). Willow leaf litter was collected in trays during the months of litter fall, July–December, to quantify leaf biomass. Leaf litter was oven-dried at 80 °C for 48 h, weighed and extrapolated from tray to tonnes per hectare. Litter decomposition was measured over 2 years in SRC willow. Mesh bags (20 × 10 cm; 1 mm aperture) each containing 5 g leaf litter (picked green leaves) were placed by each of the GHG chambers in November 2011. Bags were collected at several points postinsertion – 2 weeks then 1, 2, 4, 6, 9, 12, 18 and 24 months. Leaf litter was gently washed with distilled water, then dried at 85 °C for 24 h before dry weight was recorded.

Aboveground biomass was estimated in the grassland from four randomized plots by cutting all biomass within a 50 × 50 cm quadrat with hand shears flush to ground. Samples were taken twice during the experiment, in August 2013 and August 2014 prior to the mowing of the field. Samples were oven-dried at 80 °C for 48 h, weighed and extrapolated from quadrat to tonnes per hectare.

Belowground biomass. Belowground biomass in SRC willow was estimated using equations for aboveground stool and stem and belowground biomass found in Pacaldo *et al.* (2013a). Then, the ratio of belowground : aboveground (i.e. aboveground stool + stem) was calculated as 0.99 for our site. In the grassland, belowground biomass was measured using 5 cm diameter auger and taken at three depths (0–10, 10–20 and 20–30 cm) across four randomized plots. Roots were sieved consecutively through sieves of decreasing mesh size (3350, 2000 and 500 µm), oven-dried at 85 °C for 24 h, weighed and extrapolated from core to tonnes per hectare. Total biomass was calculated by summing total above ground biomass and belowground biomass; for SRC willow the aboveground components included stem, stool, branches and leaf biomass. Net primary production was calculated on an annual basis using two consecutive biomass measurement data sets (nondestructive for SRC willow and destructive biomass harvest for grassland). Standard error was calculated for all components of biomass, as well as for NPP.

Eddy covariance measurements

Eddy covariance towers were installed in SRC willow in April 2012 and in grassland in August 2012 to measure ecosystem CO₂ fluxes. Each system consisted of an open path infrared gas analyser (Li-7500A; Licor, Lincoln, NE, USA) and a sonic

anemometer (Windmaster Pro; Gill, Hampshire, UK). Data were logged at 20 Hz to an industrial grade USB stick in the LiCor interface box. Instrument height was 2.5 m from the ground for the grassland site. For SRC willow, instrument height was 8 m at the start of the experiment and extended as the crop grew to a maximum instrument height of 9.3 m in March 2014. After harvest, the instrument height was reduced to 3.6 m aboveground level.

Eddy covariance data were processed using EddyPro (Licor) and averaged over 30-min intervals. The applied methodology was based on the EuroFlux protocol (Aubinet *et al.*, 2000). Data were then elaborated and quality-checked using Stata IC 10 (StataCorp LP, College Station, TX, USA). Most of the data were discarded during night-time as the assumptions using for eddy covariance measurements (i.e. turbulence) were not fulfilled. Data were rejected when fluxes came from outside the flux footprint which was between 135° and 262° for SRC willow and 140–290° for grassland. Data were also discarded during rain and fog. Energy balance closure at each site was estimated only using measured data. Gapfilling to estimate Net Ecosystem Exchange (NEE) and flux partitioning into Ecosystem Respiration (R_{eco}) and Gross Primary Production (GPP) were done according to the standard methodology used in Fluxnet (<http://www.bgc-jena.mpg.de/~MDIwork/eddyproc/>; Reichstein *et al.*, 2005). NEE uncertainty (i.e. standard deviation) was computed according to the FLUXNET methodology using the online software, whilst error terms were unable to be calculated for R_{eco} and GPP as these are modelled terms.

Ecosystem GHG balance

A conceptual model was constructed to represent the whole system GHG balance for both grassland and SRC willow for two whole years during the measurement period, January 2013–December 2014 (Fig. S1, blue box). All gas flux data were expressed as g C m⁻² yr⁻¹ and soil storage terms presented as standing stock (g C m⁻²). The terminology used is as defined by Chapin *et al.* (2006); however, we assigned a positive sign to emissions of carbon to the atmosphere and a negative sign to an uptake of C by the ecosystem, as generally used in micrometeorology. Briefly, NEE was defined as the CO₂ exchange between the ecosystem and the atmosphere, measured using the eddy covariance technique. Gross Primary Productivity was defined as the fixation of carbon by autotrophic organisms and Ecosystem Respiration (R_{eco}) is the net respiration by both autotrophic and heterotrophic organisms in the ecosystem. Soil respiration is the sum of CO₂ respired by roots (autotrophic respiration) and by microbes (heterotrophic respiration). Net Primary Production is the accumulation of biomass within the study system, measured used in litter fall and biomass estimates. Net Biome Production (NBP) describes the difference between Net Ecosystem Productivity (NEP, negative sign of NEE; Reichstein *et al.*, 2012) and the carbon removed through harvest. The sign of NBP is opposite to measures described above, where negative indicates a release to the atmosphere and a positive value indicates an uptake by the ecosystem. NBP was only calculated for SRC willow where harvested biomass was removed from the system.

Statistical analysis

A basic *t*-test was performed to detect any significant difference in soil carbon stocks at 0–30 cm ($n = 15$) and 0–100 cm ($n = 3$) between land uses, using SigmaPlot 12.5. All statistical analyses for GHG and eddy data were conducted in the R programming environment (R version 3.1.3; R Core Team, 2015). GHG data were analysed using linear mixed models (Bates *et al.*, 2014) where fixed effects were treatment, year, soil temperature and soil moisture. Air temperature and soil temperature exhibited collinearity so could not both be included in the model. Chamber number was used as a random factor to account for repeat sampling over time. Main effects were tested in addition to all second-order interactions. Analysis of N₂O and CH₄ reveals normality of residuals and homoscedasticity; however, there was heteroscedasticity detected in the CO₂ data; therefore, log-transformation was performed. Model selection was performed according to Crawley (2007) using AIC to construct the minimum adequate model (see Table S2).

For eddy covariance data, a global model was constructed to assess the effects of land use and climate variables [fixed effects: treatment, photosynthetically active radiation (PAR), wind speed, rain, soil temperature, relative humidity (RH) and soil water content; random effect: date] on NEE using daily averaged data (see Table S3 for full model). There was collinearity between air temperature and soil temperature so only one was used in the model, likewise for soil water content at both depths. Data were then partitioned by site and two separate models were constructed for each data set to see whether the drivers of NEE differed between fields. The aim of this analysis was to try to identify the drivers of NEE to environmental variables which were measured on site.

Results

Weather patterns

Air temperature in 2012 were close to average values for the region. Spring of 2013 was cooler than average, whereas winter 2013 and spring of 2014 experienced higher than average temperatures (Fig. 3). The spring/summer of 2012 and winter 2013 are notably wet years with above average rainfall for the region, whilst in contrast the spring and summer of 2013 were drier than average (Fig. 3). Air temperature in 2013 was cooler and much drier than both 2012 and 2014 with an average air temperature of 9.9 °C and rainfall of 673.3 mm. 2012 was slightly cooler but wetter (10.6 °C and 1318 mm) than 2014 which experienced an average temperature of 11.1 °C and 1023 mm rainfall.

Net primary production

Total aboveground biomass in SRC willow increased from the first measurement, March 2012, to the final

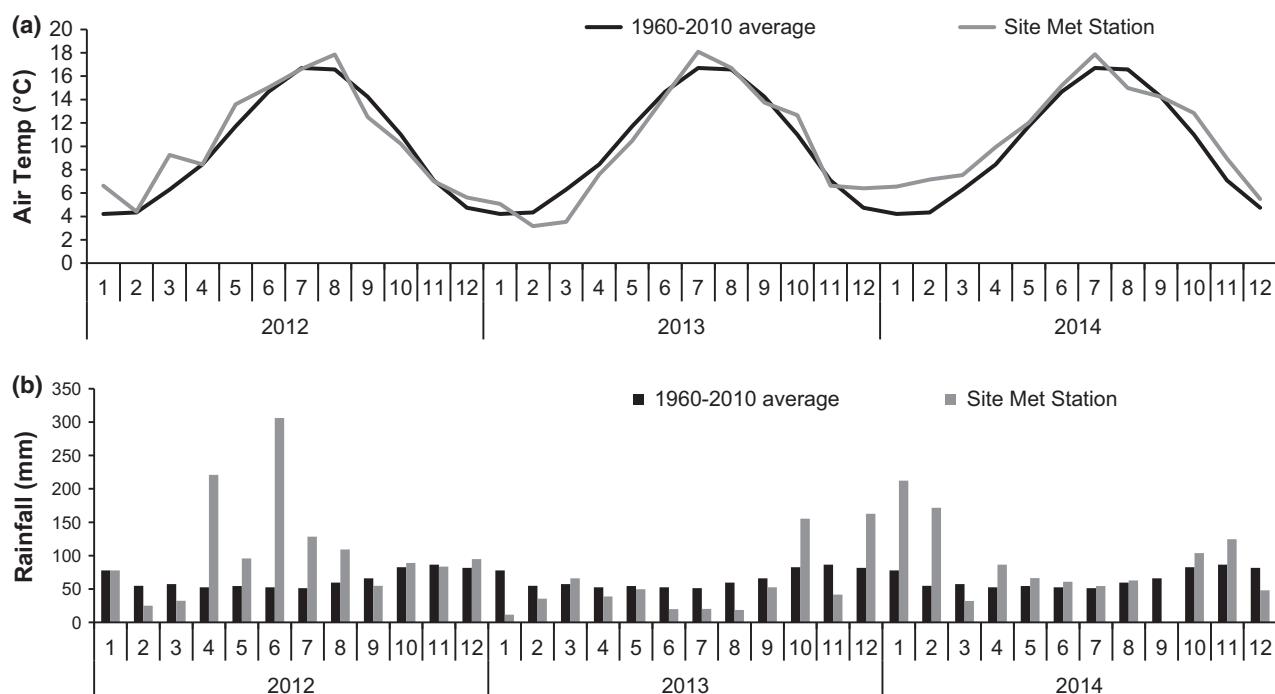


Fig. 3 (a) Monthly mean values of air temperature ($^{\circ}\text{C}$) showing 50-year average (1960–2010; black line) and values measured by site meteorological station (grey line). (b) Sum of rainfall (mm) for 50-year average (1960–2010; black line) and measured on site (grey line).

measurement before the harvest, November 2013 (Fig. 4a). Biomass was rapidly accumulated after the harvest in April 2014 with total aboveground woody biomass reaching $11.4 \pm 1.1 \text{ t ha}^{-1}$ (mean \pm SE; $n = 8$) by the end of 2014. Leaf litter was similar for 2012 and 2013 with 5.6 ± 0.2 and $5.8 \pm 0.2 \text{ t ha}^{-1} \text{ yr}^{-1}$, respectively. There was a decrease in leaf litter fall after the harvest in 2014 with only $2.1 \pm 0.2 \text{ t ha}^{-1} \text{ yr}^{-1}$. The majority of SRC willow leaf litter decomposed within the first year, with only 17% leaf litter remaining after 12 months and only 8% remaining after 2 years (Fig. S2). Total grassland biomass was over double that in 2014 compared to 2013, for both aboveground and belowground biomass (Fig. 4b). Total biomass in 2013 was higher in SRC willow ($96.2 \pm 3.6 \text{ t ha}^{-1}$; $n = 4$) than grassland ($8.7 \pm 1.5 \text{ t ha}^{-1}$), and owing to the remaining belowground biomass, total biomass remained higher in SRC willow in 2014 after harvest (69.8 ± 2.8 and $20.8 \pm 1.6 \text{ t ha}^{-1}$ for SRC willow and grassland, respectively). There was a decrease in NPP in SRC willow from 2012 to 2013, which corresponds to year 4 and year 5 of the rotation (Fig. 4c; 14.6 ± 2.1 and $10.8 \pm 2.4 \text{ t C ha}^{-1} \text{ yr}^{-1}$, respectively). There was an increase in NPP postharvest to $12.4 \pm 0.8 \text{ t C ha}^{-1} \text{ yr}^{-1}$ (Fig. 4c). In 2014, the NPP in grassland ($4.9 \pm 1.0 \text{ t C ha}^{-1} \text{ yr}^{-1}$) was less than that of SRC willow, $12.4 \pm 0.8 \text{ t C ha}^{-1} \text{ yr}^{-1}$ (Fig. 4c).

Soil respiration

CO_2 accounted for the majority of soil GHG flux, c.96% and c.99% for grassland and SRC willow, respectively. Mean soil respiration (2012–2014) was significantly higher in grassland ($1522 \pm 39 \text{ g C m}^{-2} \text{ yr}^{-1}$; mean \pm SE; $n = 8$) than in SRC willow ($912 \pm 42 \text{ g C m}^{-2} \text{ yr}^{-1}$; Fig. 5, Table S4, $P = 0.03$). Year, soil temperature and soil moisture were all significant factors affecting soil respiration ($P < 0.001$), as well as second-order interactions for treatment and year ($P < 0.001$), treatment and soil temperature ($P < 0.001$), and year and soil moisture ($P = 0.007$). According to the continuous soil respiration measurements (also reported in Ventura *et al.*, 2015), heterotrophic respiration accounted for 84% of total soil respiration in the SRC willow.

Eddy flux measurements

For the eddy covariance data, after quality control checks and footprint analysis the data remaining were 40% for grassland and 37% for SRC willow in 2013. In 2014, the remaining data for each site was 46% and 20% for grassland and SRC willow, respectively. The energy balance closure for the sites, based on measured data only, was a 73% for grassland (Fig. 6a) and 77% for SRC willow (Fig. 6b).

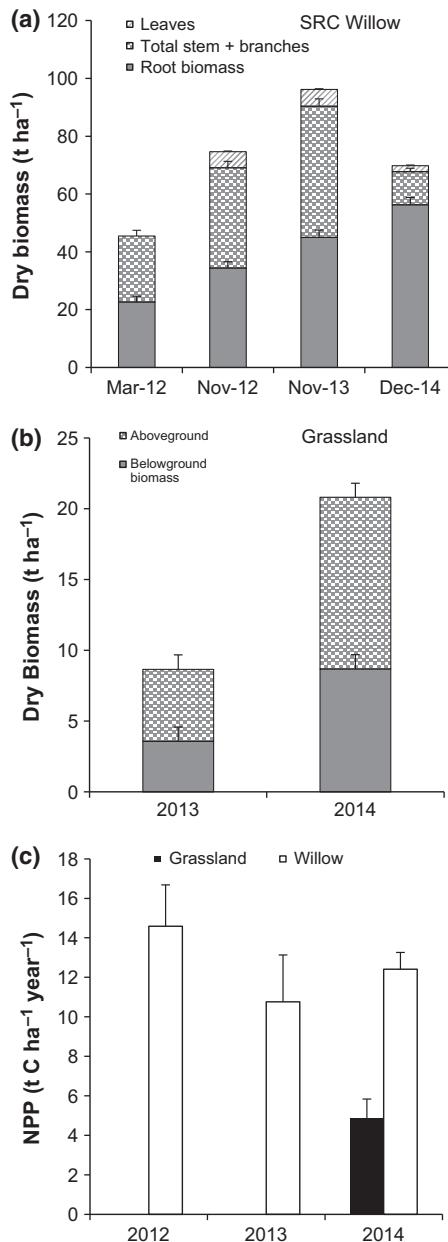


Fig. 4 (a) Total biomass for short rotation coppice willow ($t\text{ ha}^{-1}$; mean \pm SE; $n = 8$) including measured stem (checkerboard hatching) and leaf biomass (diagonal hatching), and estimated root biomass (grey fill). (b) Grassland biomass from measured aboveground (checkerboard hatching) and below-ground sampling (grey fill) ($t\text{ ha}^{-1}$; mean \pm SE; $n = 4$). (c) Net primary productivity for short rotation coppice willow (white bars) and grassland (black bars).

For grassland, the mean NEE over 2 years (2013–2014) was $119 \pm 10 \text{ g C m}^{-2} \text{ yr}^{-1}$ (mean \pm SD). In year one (2013), the grassland was a net source of carbon, $246 \pm 11 \text{ g C m}^{-2} \text{ yr}^{-1}$, whereas in year two (2014) it was a net sink, $-9 \pm 16 \text{ g C m}^{-2} \text{ yr}^{-1}$. In year one,

there was a small uptake of carbon during the growing season from June 2013 to the end of July 2013 (Fig. 7a); however, in year two there is a more defined uptake period starting from March 2014. This early onset of carbon fixation could be attributed to the higher mean monthly temperature in January–March 2014 compared to 2013 (Fig. 3). SRC willow was a C sink for the 2-year duration of the experiment with a mean annual NEE of $-620 \pm 18 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Fig. 7b). In the first year, which corresponded to the 4th year of growth, the site was a large sink of carbon ($-901 \pm 23 \text{ g C m}^{-2} \text{ yr}^{-1}$). The NEE for the second year was smaller due to the harvest in April 2014 ($-339 \pm 27 \text{ g C m}^{-2} \text{ yr}^{-1}$). NEE was lower in the SRC willow than in grassland during the second year ($P < 0.001$). Analyses of eddy covariance data also revealed that NEE in grassland and SRC willow were driven by different components (Table S3). In the grassland, PAR, year, soil (and air) temperature, wind speed and rain were factors affecting NEE, whilst in the SRC willow only PAR, year and soil water content were affecting NEE. Relative humidity was not found to be a factor affecting NEE at either site.

There were also differences in R_{eco} and GPP between grassland and SRC willow. R_{eco} was 33% higher in 2014 than in 2013 in grassland (1261 and $1675 \text{ g C m}^{-2} \text{ yr}^{-1}$ for year one and year two, respectively). R_{eco} in SRC willow in year one was lower than both years in grassland at $971 \text{ g C m}^{-2} \text{ yr}^{-1}$. In 2014, R_{eco} was larger than year one in SRC willow and both years in the grassland site at $1971 \text{ g C m}^{-2} \text{ yr}^{-1}$. Mean R_{eco} over 2 years was similar for grassland and SRC willow, 1468 and $1471 \text{ g C m}^{-1} \text{ yr}^{-1}$, respectively. GPP in grassland was 1015 and $1683 \text{ g C m}^{-2} \text{ yr}^{-1}$ for year one and two, respectively. In SRC willow, GPP was higher than the grassland for both years at 1873 and $2309 \text{ g C m}^{-2} \text{ yr}^{-1}$ for year one and year two, respectively. Over 2 years, mean GPP was higher in SRC willow than in grassland, 2091 and $1349 \text{ g C m}^{-2} \text{ yr}^{-1}$, respectively.

Belowground carbon pools

Soil carbon stocks (Table 2) were higher in the grassland than in the SRC willow for both the 0–30 and 0–100 cm profiles, but for the latter this effect was only significant to $P = 0.062$, despite a clear trend. For 0–30 cm, we found $63.4 \pm 3.5 \text{ t C ha}^{-1}$ in grassland and $42.6 \pm 1.8 \text{ t C ha}^{-1}$ in SRC willow (mean \pm SE; $t_{(28)} = -5.30$, $P < 0.001$). And for the 0–100 cm profile, there was 107.6 ± 1.8 and $77.3 \pm 7.7 \text{ t C ha}^{-1}$ for grassland and SRC willow, respectively ($t_{(4)} = -3.84$, $P = 0.062$). The grassland which was used initially for chamber measurements had a similar carbon stocks to grassland in the upper 30 cm ($61.2 \pm 2.8 \text{ t C ha}^{-1}$),

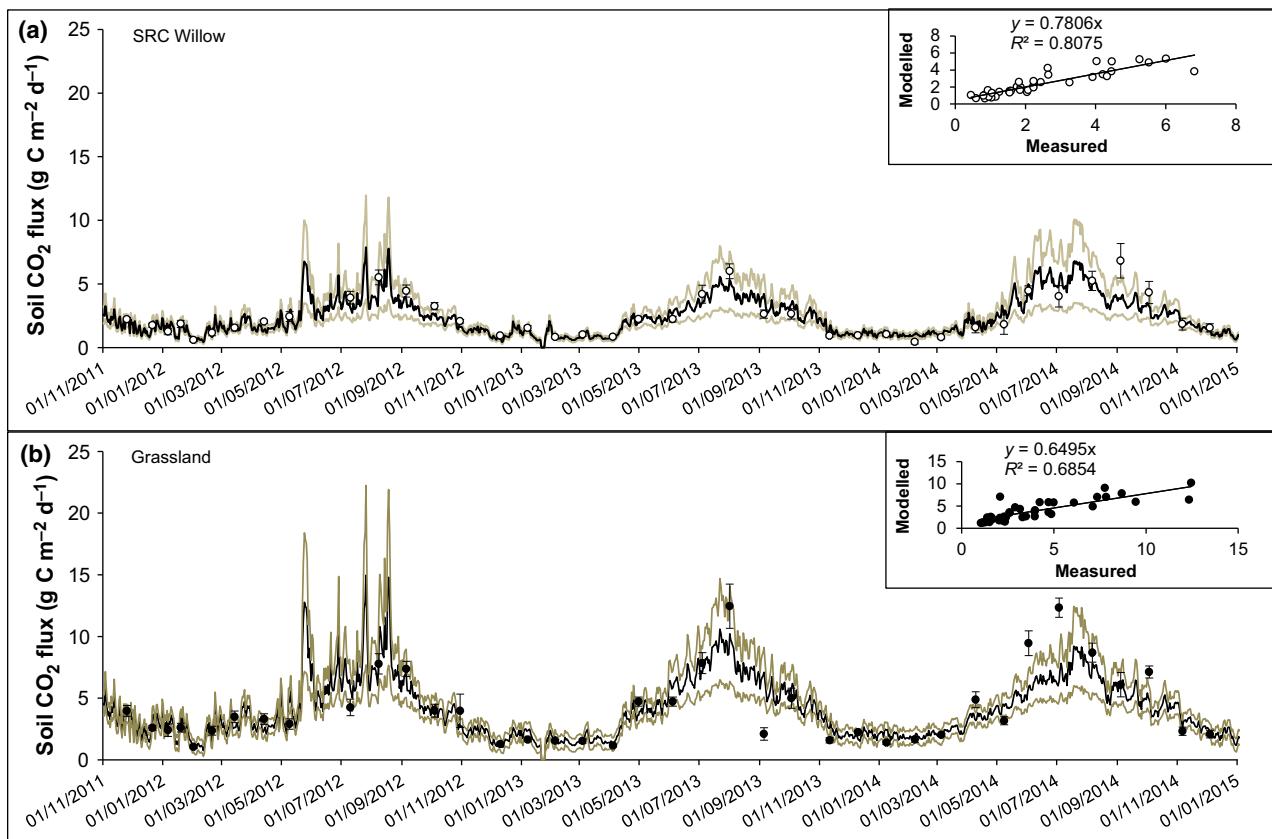


Fig. 5 Soil CO_2 flux ($\text{g C m}^{-2} \text{ day}^{-1}$) for short rotation coppice willow (a; white circles) and grassland (b; black circles) ($n = 8$). Periodic sampling events (circles) and modelled CO_2 flux (black line; using soil temperature) are shown. Green line indicates 5th and 95th percentiles around the modelled values. Additionally, modelled CO_2 data are regressed against measured CO_2 data for both sites and the relationship shown on the graph as R^2 .

with slightly less carbon in the 100 cm profile than the SRC willow ($63.8 \pm 4.1 \text{ t C ha}^{-1}$; Table 2).

Soil GHG fluxes

N_2O and CH_4 were not important contributors to the whole GHG balance of these two particular sites, accounting for less than 4% (3.4% N_2O and 0.4% CH_4) for grassland and less than 1% (0.77% N_2O and 0.07% CH_4) for SRC willow. Mean N_2O fluxes at both sites (2012–2014) were very low (within detection limit of equipment) with emissions of 1.2 ± 0.3 and $1.9 \pm 0.6 \text{ g C m}^{-2} \text{ yr}^{-1}$ for grassland and SRC willow, respectively (Fig. S3; 4.4 ± 1.1 and $7.0 \pm 2.2 \text{ g CO}_2\text{-eq m}^{-2} \text{ yr}^{-1}$ for grass and SRC, respectively). There was no difference between N_2O fluxes between the sites ($P = 0.81$; Table S5). N_2O flux was significantly affected by year across both sites ($P = 0.003$), as well as an interaction between year and soil moisture ($P = 0.007$). CH_4 was also very low at both sites; however, there was a difference between the sites with an

emission of $0.2 \pm 0.2 \text{ g C m}^{-2} \text{ yr}^{-1}$ from grassland and uptake of $-0.2 \pm 0.1 \text{ g C m}^{-2} \text{ yr}^{-1}$ in SRC willow ($P = 0.003$; Table S6, Fig. S4; 0.7 ± 0.7 and $-0.7 \pm 0.4 \text{ g CO}_2\text{-eq m}^{-2} \text{ yr}^{-1}$ for grass and SRC, respectively). For both sites, soil temperature significantly affected CH_4 flux ($P < 0.001$), as well an interaction between soil moisture and soil temperature ($P = 0.02$).

Conceptual model

Data from January 2013 to December 2014 were summarized in a conceptual model to allow comparison of the grassland and SRC willow (Fig. 8). This figure shows the movement of carbon through the ecosystem, highlighting major fluxes and stocks. The harvested carbon was expressed on annual basis (i.e. total harvested biomass was divided by the rotation length in the willow) and is shown, 445 ± 68 and $399 \pm 23 \text{ g C m}^{-2} \text{ yr}^{-1}$ (mean \pm SE) for grassland and SRC willow, respectively. However, as the mowed grass was not removed

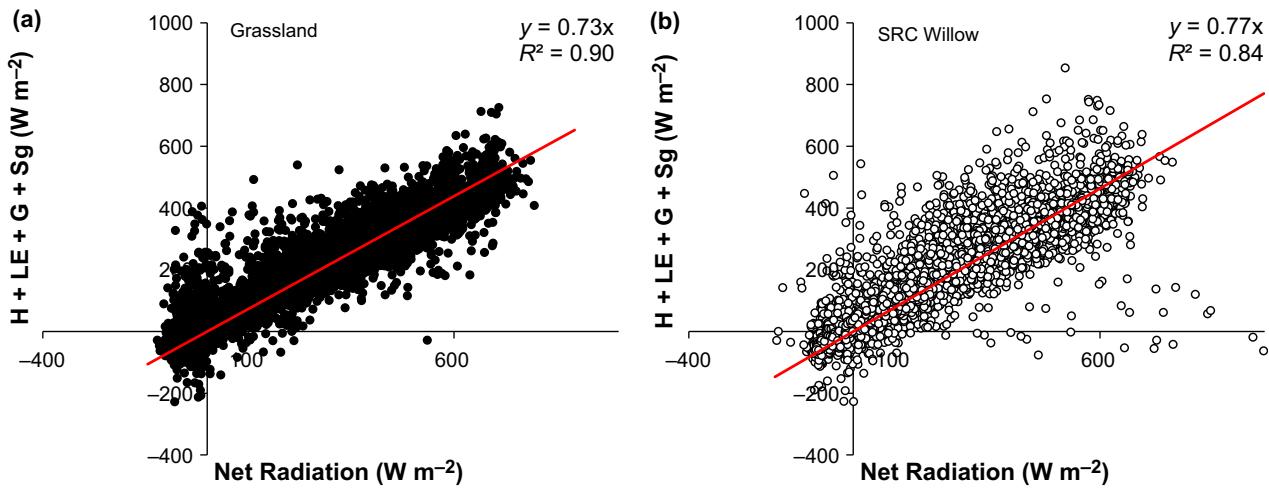


Fig. 6 Energy balance closure for grassland (a; black circles) and short rotation coppice willow (b; white circles) for 2013–2014, where H is sensible heat flux, LE is latent heat flux, G is soil heat flux and Sg is soil heat storage. Strength of regression indicated on graph by R^2 value.

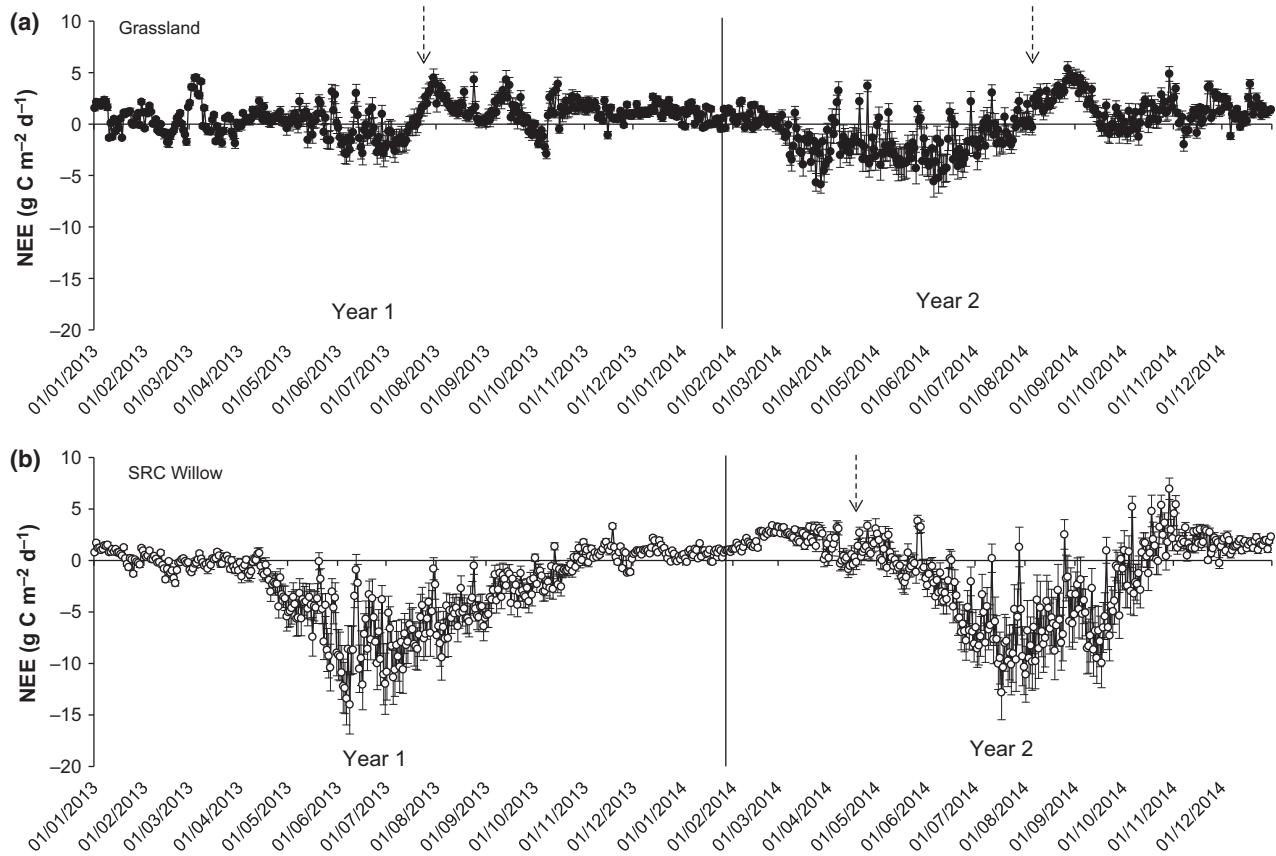


Fig. 7 Net Ecosystem Exchange (NEE; g C m⁻² day⁻¹; mean \pm SD) for grassland (a; black circles) and short rotation coppice willow (b; white circle) for 2013–2014. Harvest events at both sites are indicated by dashed arrows.

from the site, NBP is equal to NEE. Thus, mean NBP (2013–2014) was -118 ± 10 g C m⁻² yr⁻¹ for grassland and 221 ± 66 g C m⁻² yr⁻¹ for SRC willow

(mean \pm SD), which, despite the removal of 399 g C m⁻² yr⁻¹ biomass from the SRC field, remained a net sink for carbon.

Impact of harvest in SRC willow

The SRC willow was harvested in April 2014 which corresponded to year 5 of the first rotation. There was no detectable effect of the harvest on soil moisture or soil temperature in the SRC willow, compared to preharvest measurements. The effect of the harvest on the NEE can be seen in Fig. 7b (dashed arrow indicated harvest date), where NEE decreased prior to harvest and then quickly increased after harvest. The smaller NEE and increased R_{eco} observed in SRC willow in 2014 compared to 2013 is likely attributable to the disturbance

Table 2 Soil carbon stocks (t C ha^{-1}) under grassland and short rotation coppice willow, calculated on an equivalent soil mass basis, for 0–30 cm and 0–100 cm. Initial grassland refers to site where static chamber measurements were taken prior to installation of eddy covariance monitoring equipment. Samples collected in October 2012. $n = 15$ for 0–30 samples and $n = 3$ for 0–100 cm samples

Soil depth (cm)	Grassland	SRC willow	Initial grassland
	Mean \pm SE (t C ha^{-1})		Mean \pm SE (t C ha^{-1})
0–30	63.4 ± 3.5	$42.6 \pm 1.8^*$	61.2 ± 2.8
0–100	107.6 ± 1.8	77.3 ± 7.7 (10%)	63.8 ± 4.1

*Significance to 0.05 (5%) and significance to 0.1 (10%).

caused by the harvest. The site quickly became a net C sink again as there was a rapid re-sprout of willow stumps and understory vegetation. There was no noticeable effect on soil CO_2 and CH_4 emissions as a result of the harvest. There was a large one-off emission of N_2O in June 2014, 2 months postharvest, which may have arisen as a result of the harvesting process (Fig. S3b).

Discussion

Understanding the consequences of LUC for ecosystem GHG balance is important if we are to tackle the impact of agricultural practices on global GHG emissions. This research addressed a critical – the provision of empirical GHG balance data from commercial-scale operations, where bioenergy has been deployed for a period of years. It has demonstrated that over a 2-year period (including the harvest operation in SRC willow), during a side-by-side commercial-scale comparison, an SRC willow field was a net sink for carbon, whilst the minimally managed grassland field was a net source for carbon. N_2O and CH_4 emissions were generally low for both sites, contributing little to the total GHG balance for these contrasting land-use types in southern England. Thus, we can conclude that 7 years postland-use transition, this SRC bioenergy crop had an improved GHG balance relative to the adjacent grassland. This

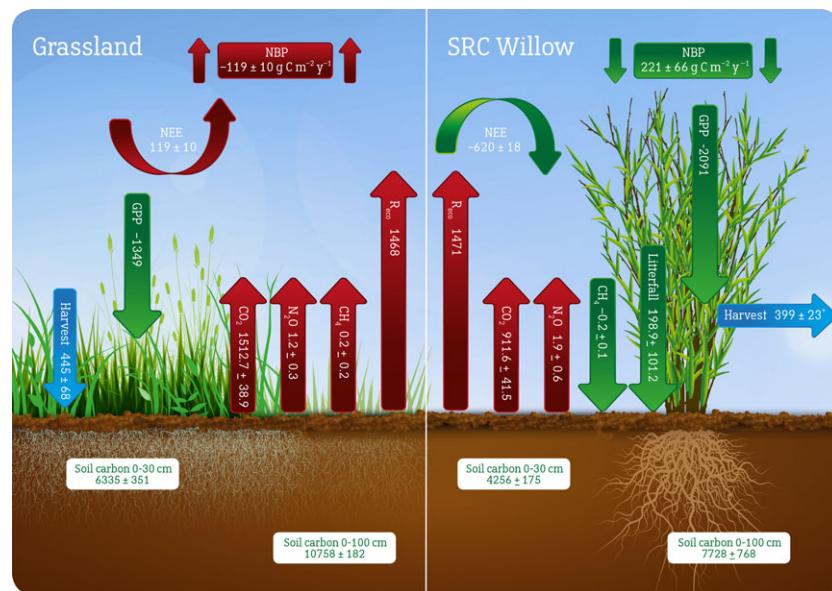


Fig. 8 Annual greenhouse gas budget for grassland and short rotation coppice willow for measurement period January 2013 to December 2014. All fluxes are in $\text{g C m}^{-2} \text{yr}^{-1}$ in square boxes and soil storage terms presented as standing stock (g m^{-2}) in oval boxes. Measured values are presented as mean \pm SE, except for net ecosystem exchange (NEE) and Net Biome Production (NBP) where measured values are presented as mean \pm SD (see Materials and methods for details on uncertainty calculation for NEE). Note for all fluxes, apart from NBP, a negative flux indicated a gain to the ecosystem and a positive flux indicates a loss to the atmosphere.

¹Harvest data have been annualized from the total biomass taken off the field during coppicing at year 5.

suggests that not only did this LUC provide bioenergy as a net provisioning ecosystem service, but was also able to contribute to improved climate regulation through the generation of a net carbon sink relative to the original land use. In the area of bioenergy science, this is an important empirical finding and suggests that in temperate climates, where reasonable land-use transitions are considered, bioenergy may add positively to the multifunctional landscape, as suggested recently by those such as Manning *et al.* (2015) and Souza *et al.* (2015). These results coupled with the potential carbon and GHG savings made by replacing fossil fuels demonstrate the potential of bioenergy for climate change mitigation and improved energy security (Cannell, 2003; Styles & Jones, 2007).

Improved grasslands are important sources of terrestrial carbon storage, holding the second largest store after bogs, with approximately 274×10^6 t C (Ostle *et al.*, 2009) and here we hypothesized that LUC from grassland to SRC willow would lead to a significant reduction in GHG emissions as proposed in previous modelling studies in this temperate bioenergy system (Hillier *et al.*, 2009; Hastings *et al.*, 2014; Milner *et al.*, 2015). In a UK context, conversion of semipermanent, permanent or managed grassland to bioenergy cropping systems represents one of the most significant potential land-use transitions, since grassland is a considerable part of the UK landscape (4.5×10^6 ha; DEFRA *et al.*, 2007) and because management of grasslands can vary widely in the UK, particularly with respect to fertilizer input and grazing. This can have a dramatic effect on consequential GHG and carbon balance as a result of LUC. For the grassland studied here, we found that over a 2-year measurement period, grassland was a net source for carbon and SRC willow was a net sink. Even when considering the carbon removed from the system scaled on an annual basis in harvest biomass (NBP), the SRC willow site remained a sink for carbon. In this experiment, we observed high biomass yields for SRC willow, comparable to those found in some other studies (Laurent *et al.*, 2015) but within the range reported by Allwright & Taylor (2016). To our knowledge, there has been only one previous limited study of eddy covariance measurements over SRC willow for bioenergy (Drewer *et al.*, 2012), though much research attention has been focussed on SRC poplar. These studies have generally found that SRC poplar is a sink at the ecosystem level (Arevalo *et al.*, 2011; Jassal *et al.*, 2013; Sabbatini *et al.*, 2015), even as soon as 2 years postestablishment of the crop (Verlinden *et al.*, 2013).

One question from our study is the relevance of the grassland considered here, since retention of cut grass on the surface, which resulted in no C exports from the system, could be considered uncommon with grazing

and mowing for hay or silage much more likely as a management option (Smit *et al.*, 2008). As a result, grassland NBP was equal to NEE at -119 ± 10 g C m $^{-2}$ yr $^{-1}$ at our site. Qun & Huizhi (2013) investigated similarly managed grassland with no exports of carbon and found that the site was similarly a net source of carbon, with a NBP of -138 g C m $^{-2}$ yr $^{-1}$. Thus, we can conclude that management of the grazing and mowing regime might be central to the carbon balance of such a system and determine net source or sink status. We identified PAR and soil moisture to be the main climatic drivers of NEE in grassland and SRC willow, which has been found in other studies (Ruimy *et al.*, 1995; Qun & Huizhi, 2013; Shao *et al.*, 2015). In contrast, some studies have identified leaf area index (LAI) to be the main biophysical driver of NEE in SRC poplar (Broeckx *et al.*, 2014; Zenone *et al.*, 2015), but our data for willow do not support this. Data syntheses from a network of sites such as FLUXNET have already begun identifying driving factors of NEE, GPP and R_{eco} over a number of biomes (Law *et al.*, 2002), and as the amount of flux data from bioenergy crops increases, there is potential for syntheses in these biomes in future.

In this experiment, we found that CO₂ was the main contributor to soil GHG emissions in both sites, supporting the observations of Drewer *et al.* (2012) who also found CO₂ to be the dominant soil GHG for SRC willow at a second UK site. In the SRC willow, we were able to observe the partitioning of soil CO₂ flux which revealed that 84% of total soil respiration was heterotrophic in origin (Ventura *et al.*, 2015). Since heterotrophic respiration can vary from 10% to 90% depending on vegetation type and time of year (Hanson *et al.*, 2000), our data fall within this wide range. Future work at this site should measure autotrophic and heterotrophic respiration in grassland for a direct comparison and inference on the effects of LUC to bioenergy.

Grasslands can vary in both space and time for GHG emissions and carbon balance (Soussana *et al.*, 2007; Imer *et al.*, 2013), as found here where the grassland in this study was a net source of carbon in 2013 and a net sink in 2014, possibly attributable to the higher temperatures observed in January–March 2014 compared to 2013. Grass begins growing when air temperature exceeds 5 °C (Robson *et al.*, 1988), which was achieved earlier in 2014, providing an extended season for carbon fixation. This combination of increased temperature with an increase in winter rainfall (which resulted in increased soil moisture) could explain the higher above-ground biomass in grassland and consequently why the site was a net sink in 2014 (Pitt & Heady, 1978).

As well as large variability, there are also large uncertainties surrounding the overall GHG balance of

temperate grasslands (Janssens *et al.*, 2003). Within the literature, there are reports that grasslands are acting as both carbon sources and carbon sinks (Scurlock & Hall, 1998; Bellamy *et al.*, 2005; Soussana *et al.*, 2007; Ciais *et al.*, 2010; Merbold *et al.*, 2014; Schipper *et al.*, 2014; Rutledge *et al.*, 2015), with the balance tightly linked to management regime, including fertilizer application, rotation and grazing regime (Smith, 2014), with changes in management causing grasslands to switch from a source to a sink (Merbold *et al.*, 2014). Grassland management practices such as fertilization, grazing and mowing lead to large N₂O and CH₄ emissions which counterbalance this CO₂ sink (Ciais *et al.*, 2010; Imer *et al.*, 2013). For our particular site, N₂O and CH₄ contributed little to GHG balance of either land use and both were present in small quantities. Interestingly, grassland was a net source of CH₄, whereas SRC willow was a net sink, but the fluxes were small. SRC willow has been found to be a net sink for CH₄ in other studies to a similar extent to that found here (Drewer *et al.*, 2012; Kern *et al.*, 2012). For both sites, there was an effect of soil moisture, and a significant interaction for soil moisture and soil temperature on CH₄ fluxes, confirming a number of other studies in bioenergy crops (Drewer *et al.*, 2012; Kern *et al.*, 2012) and grasslands (Kammann *et al.*, 2001; Imer *et al.*, 2013). Future climate changes may result in the need for fertilizer to maintain yields in SRC willow, which may lead to an altered GHG balance due to subsequent N₂O emissions.

Here, we found that grassland had significantly higher soil carbon stocks than the SRC willow up to 30 cm with a similar trend at 1 m depth. Sampling depth is a recurrent problem in studies which attempt to quantify soil carbon (Dolan *et al.*, 2006; Blanco-Canqui & Lal, 2008) and it is essential that the whole profile is sampled to draw robust conclusions (Harrison *et al.*, 2011). At this particular site, the higher soil carbon observed in grassland may be attributable to the amount of organic material left on the soil surface after mowing (Post & Kwon, 2000) and may not be widely representative of much managed rotational grassland. In grassland, on average, $445 \pm 48 \text{ g C m}^{-2} \text{ yr}^{-1}$ of organic material was left on the soil surface after mowing however in SRC willow, annual litter fall reached a maximum of $292 \pm 12.5 \text{ g C m}^{-2} \text{ yr}^{-1}$ in 2013. There have been reports in the literature of both increased soil carbon under SRC compared to grassland (Zan *et al.*, 2001; Arevalo *et al.*, 2009), as well as others which have found no significant difference (Grigal & Bergeson, 1998; Walter *et al.*, 2015). Walter *et al.* (2015), from a chronosequence of SRC sites, suggested that this transition results in a redistribution of carbon through the profile, despite total SOC stock not being significantly different. After 7 years postconversion, we may be

beginning to see redistribution of C in the soil profile. We found that at the two grasslands sites 59% and 96% carbon was stored in the top 30 cm, whereas in SRC willow 54% carbon was stored in the top 30 cm of the whole 100 cm profile. Whilst these differences are not large, the transition may still be at the early stages of C redistribution through the soil profile, though further data would be required to confirm this postulation. Chronosequence data also suggest that after initial conversion from grassland to SRC willow, there can be a loss of soil carbon for up to 5 years, which is followed by recovery up to 19 years (Pacaldo *et al.*, 2013b). Our site is only 7 years postconversion and therefore is likely still in the recovery phase with respect to soil carbon.

One limitation of this study is the lack of measured root biomass in the SRC willow system, which may have resulted in an underestimation of the SRC willow sink postharvest. However, the calculated values in this study are in line with empirical findings recently published by Cunniff *et al.* (2015); therefore, we are able to use these estimated with some confidence. This demonstrates one of the challenges of working in a commercial system where restrictions to experimental measurements are imposed by the commercial regime.

Capturing the effects of a commercial harvest on the soil and ecosystem GHG balance was important since harvesting is recognized as one of the most energy intensive stages of the SRC willow life cycle due to the large consumption of diesel fuel (Murphy *et al.*, 2014) and relatively little is known about the effects on the GHG balance in SRC willow (Vanbeveren *et al.*, 2015). From our study, we have shown that whilst there is an increase in R_{eco} and subsequently NEE after the harvest, within 3 weeks of harvest, the site was returned to being a sink for carbon. The observed increase in NEE is comparable to that observed by Zenone *et al.* (2015) for the 2nd year postestablishment of an SRC poplar plantation; indicating the effect of disturbance on NEE. LCA findings have shown that whilst the harvest can increase emissions due to the harvest machinery, the carbon sink created by SRC willow is able to offset these emissions and result in a negative GHG balance (reported in the range of -138 to -53 kg CO₂-eq. per odt biomass; Caputo *et al.*, 2014). We also observed a one-off peak in N₂O emissions, 2 months postharvest, which was the largest emission, observed across both sites for the duration of the experiment. In contrast, other studies have observed little effect of harvest on N₂O emissions from SRC cultures (Zona *et al.*, 2013b). It is possible that this emission arose as a result of increased soil exposure after harvest and increased rainfall in May and June 2014, relative to 2013. It is also possible that there was some compaction due to the harvest

machinery which can cause a reduction in soil porosity, in turn resulting in increased N₂O emissions. Soil N₂O fluxes are known to vary spatially and temporally and to arise quickly after changes in rainfall, temperature and management (Skiba & Smith, 2000). N₂O emissions, therefore, require more intense monitoring to be able to capture these emissions, since a single large emission can account for a large proportion of total N₂O fluxes over a measurement period (Zona *et al.*, 2013b).

In conclusion, we have shown that LUC to SRC willow from grassland can result in reduced GHG emissions. In the minimally managed site studied here, where harvested grass remained on the field, we found that grassland was a net carbon source and SRC willow a net carbon sink, 7 years after land conversion. However, soil carbon stocks were likely still in recovery as soil C at the SRC site remained significantly lower than grassland, even after this amount of time postestablishment. Whilst grasslands have been shown to be highly variable, there is evidence that this LUC may result in climate mitigation advantages and may be considered a viable bioenergy option for the future.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Timeline of experimental measures taken in grassland (G, green) and SRC willow (W, brown).

Figure S2. Percentage mass loss from leaf litter decomposition in SRC willow over 24 months.

Figure S3. Soil N_2O flux ($\text{mg N}_2\text{O m}^{-2} \text{ day}^{-1}$) for (a) grassland, (b) SRC willow and (c) both sites where grassland is shown as black circles and SRC willow is white circles. Monthly sampling took place from eight chambers per field, standard error shown.

Figure S4. Soil CH_4 flux ($\text{mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$) for (a) grassland, (b) SRC willow and (c) both sites where grassland is shown as black circles and SRC willow is white circles. Monthly sampling took place from eight chambers per field, standard error shown.

Table S1. Root and stone content of grassland, SRC willow and initial grassland. $n = 15$ for 0–30 cm cores and $n = 3$ for 0–100 cm cores.

Table S2. Model selection: variables included in linear mixed models developed to explain variation in soil GHG flux.

Table S3. Output table of linear mixed models statistics on net ecosystem exchange data.

Table S4. Output table for minimum adequate model for soil CO_2 flux.

Table S5. Output table for minimum adequate model for soil N_2O flux.

Table S6. Output table for minimum adequate model for soil CH_4 flux.